

**WEST**[Help](#)[Logout](#)[Interrupt](#)[Main Menu](#)[Search Form](#)[Posting Counts](#)[Show S Numbers](#)[Edit S Numbers](#)[Preferences](#)[Cases](#)**Search Results -**

Terms	Documents
L3 and solfataricus	21

Database: 

US Patents Full-Text Database	▲
US Pre-Grant Publication Full-Text Database	
JPO Abstracts Database	
EPO Abstracts Database	
Derwent World Patents Index	
IBM Technical Disclosure Bulletins	▼

Search:

L4

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<u>Set Name</u> side by side	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u> result set
<i>DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ</i>			
<u>L4</u>	L3 and solfataricus	21	<u>L4</u>
<u>L3</u>	L1 and trehalose	398	<u>L3</u>
<u>L2</u>	L1 same solfataricus	10	<u>L2</u>
<u>L1</u>	amylase	16004	<u>L1</u>

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 21 of 21 returned.

☐ 1. Document ID: US 6391595 B1

L4: Entry 1 of 21

File: USPT

US-PAT-NO: 6391595

DOCUMENT-IDENTIFIER: US 6391595 B1

TITLE: Transferase and amylase, process for producing the enzymes, use thereof, and gene coding for the same

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc	Image
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☐ 2. Document ID: US 6346394 B1

L4: Entry 2 of 21

File: USPT

US-PAT-NO: 6346394

DOCUMENT-IDENTIFIER: US 6346394 B1

TITLE: Recombinant thermostable enzyme which releases trehalose from non-reducing saccharide

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc	Image
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☐ 3. Document ID: US 6303346 B1

L4: Entry 3 of 21

File: USPT

US-PAT-NO: 6303346

DOCUMENT-IDENTIFIER: US 6303346 B1

TITLE: Method of producing saccharide preparations

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc	Image
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☐ 4. Document ID: US 6284534 B1

L4: Entry 4 of 21

File: USPT

US-PAT-NO: 6284534

DOCUMENT-IDENTIFIER: US 6284534 B1

TITLE: Yeast vector comprising a shortened promoter sequence

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc	Image
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☐ 5. Document ID: US 6150153 A

L4: Entry 5 of 21

File: USPT

US-PAT-NO: 6150153

DOCUMENT-IDENTIFIER: US 6150153 A

TITLE: Thermostable trehalose-releasing enzyme

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc	Image
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☐ 6. Document ID: US 6136571 A

L4: Entry 6 of 21

File: USPT

US-PAT-NO: 6136571

DOCUMENT-IDENTIFIER: US 6136571 A

TITLE: Method of producing saccharide preparations

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc	Image
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☐ 7. Document ID: US 6129788 A

L4: Entry 7 of 21

File: USPT

US-PAT-NO: 6129788

DOCUMENT-IDENTIFIER: US 6129788 A

TITLE: Method of producing saccharide preparations

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc	Image
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☐ 8. Document ID: US 6100073 A

L4: Entry 8 of 21

File: USPT

US-PAT-NO: 6100073

DOCUMENT-IDENTIFIER: US 6100073 A

TITLE: Acid-stable and thermo-stable enzymes derived from sulfolobus species

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc	Image
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☐ 9. Document ID: US 6087149 A

L4: Entry 9 of 21

File: USPT

US-PAT-NO: 6087149

DOCUMENT-IDENTIFIER: US 6087149 A

TITLE: Starch conversion process

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc	Image
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☐ 10. Document ID: US 6027918 A

L4: Entry 10 of 21

File: USPT

US-PAT-NO: 6027918

DOCUMENT-IDENTIFIER: US 6027918 A

TITLE: Recombinant thermostable enzyme which releases trehalose from non-reducing saccharide

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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☐ 11. Document ID: US 6017899 A

L4: Entry 11 of 21

File: USPT

US-PAT-NO: 6017899

DOCUMENT-IDENTIFIER: US 6017899 A

TITLE: Non-reducing saccharide-forming enzyme, its preparation and uses

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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☐ 12. Document ID: US 5976856 A

L4: Entry 12 of 21

File: USPT

US-PAT-NO: 5976856

DOCUMENT-IDENTIFIER: US 5976856 A

TITLE: Recombinant thermostable enzyme which forms non-reducing saccharide from reducing amylaceous saccharide

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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☐ 13. Document ID: US 5922578 A

L4: Entry 13 of 21

File: USPT

US-PAT-NO: 5922578

DOCUMENT-IDENTIFIER: US 5922578 A

TITLE: Recombinant thermostable enzyme which forms non-reducing saccharide from reducing amylaceous saccharide

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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☐ 14. Document ID: US 5863771 A

L4: Entry 14 of 21

File: USPT

US-PAT-NO: 5863771

DOCUMENT-IDENTIFIER: US 5863771 A

TITLE: Saccharide composition comprising maltooligosylturanose and maltooligosylpalatinose, its preparation and uses

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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☐ 15. Document ID: US 5856146 A

L4: Entry 15 of 21

File: USPT

US-PAT-NO: 5856146

DOCUMENT-IDENTIFIER: US 5856146 A

TITLE: Recombinant thermostable enzyme which releases trehalose from non-reducing saccharide

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KIMC	Draw Desc	Image
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☐ 16. Document ID: US 5723327 A

L4: Entry 16 of 21

File: USPT

US-PAT-NO: 5723327

DOCUMENT-IDENTIFIER: US 5723327 A

TITLE: Thermostable trehalose-releasing enzyme, and its preparation and uses

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KIMC	Draw Desc	Image
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☐ 17. Document ID: US 5716838 A

L4: Entry 17 of 21

File: USPT

US-PAT-NO: 5716838

DOCUMENT-IDENTIFIER: US 5716838 A

TITLE: Non-reducing saccharide-forming enzyme, its preparation and uses

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KIMC	Draw Desc	Image
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☐ 18. Document ID: US 5714368 A

L4: Entry 18 of 21

File: USPT

US-PAT-NO: 5714368

DOCUMENT-IDENTIFIER: US 5714368 A

TITLE: Thermostable non-reducing saccharide-forming enzyme its production and uses

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KIMC	Draw Desc	Image
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☐ 19. Document ID: US 5677442 A

L4: Entry 19 of 21

File: USPT

US-PAT-NO: 5677442

DOCUMENT-IDENTIFIER: US 5677442 A

TITLE: Method of crystallizing trehalose without using organic solvent

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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☐ 20. Document ID: US 5610047 A

L4: Entry 20 of 21

File: USPT

US-PAT-NO: 5610047

DOCUMENT-IDENTIFIER: US 5610047 A

TITLE: Non-reducing saccharide-forming enzyme, its preparation and uses

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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☐ 21. Document ID: EP 727485 A1

L4: Entry 21 of 21

File: EPAB

Aug 21, 1996

PUB-NO: EP000727485A1

DOCUMENT-IDENTIFIER: EP 727485 A1

TITLE: Method for conversion of a starch material, and enzyme composition suitable therefor

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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L4: Entry 5 of 21

File: USPT

Nov 21, 2000

US-PAT-NO: 6150153

DOCUMENT-IDENTIFIER: US 6150153 A

TITLE: Thermostable trehalose-releasing enzyme

DATE-ISSUED: November 21, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ikegami; Shouji	Okayama			JP
Kubota; Michio	Okayama			JP
Sugimoto; Toshiyuki	Okayama			JP
Miyake; Toshio	Okayama			JP

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Kabushiki Kaisha Hayashibara Seibutsu Kagaku Kenkyujo	Okayama			JP	03

APPL-NO: 08/ 888158 [PALM]

DATE FILED: July 3, 1997

## PARENT-CASE:

This is a continuation of parent application Ser. No. 08/485,132 filed Jun. 7, 1995, now U.S. Pat. No. 5,723,327.

## FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-166126	June 25, 1994
JP	7-109130	April 11, 1995

INT-CL: [07] C12 N 1/20

US-CL-ISSUED: 435/252.1; 435/822, 435/200, 435/201

US-CL-CURRENT: 435/252.1; 435/200, 435/201, 435/822

FIELD-OF-SEARCH: 435/252.1, 435/822, 435/200, 435/201

## PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

Search Selected

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	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/>	<u>4521252</u>	June 1985	Miyake et al.	
<input type="checkbox"/>	<u>4762857</u>	August 1988	Bollin, Jr. et al.	
<input type="checkbox"/>	<u>4839164</u>	June 1989	Smith	
<input type="checkbox"/>	<u>5026566</u>	June 1991	Roser	
<input type="checkbox"/>	<u>5455168</u>	October 1995	Maruta et al.	
<input type="checkbox"/>	<u>5472863</u>	December 1995	Maruta et al.	
<input type="checkbox"/>	<u>5723327</u>	March 1998	Ikegami et al.	435/201

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
50-154485	December 1975	JP	
58-23799	February 1983	JP	
58-72598	April 1983	JP	
58-216695	December 1983	JP	
2106912	April 1983	GB	

## OTHER PUBLICATIONS

Lama et al, Biotech. Letters 12(6):431-432, 1990.

Chemical Abstracts 117:22222K, 1992.

ATCC Catalogue of Bacteria and Bacteriophages, 18.sup.th Edition, 1992, p. 363.

"CATALOGUE OF BACTERIA AND PHAGES", Amer. Type Cult Collec., 18th Edition, p. 363, (1992).

Handbook of Amylases and Related Enzymes, Their Sources, Isolation Metods, Properties and Applications, pp. 18-63, Wheaton & Co., Ltd. (press) (1998).

Lama, L. et al. "Starch Conversion With Immobilized Thermophilic Archaeobacterium Sulfolobus Solfataricus", Biotech. Ltrs., vol. 12, No. 6, pp. 431-432 (1990).

"CATALOGUE OF FUNGI/YEASTS", Amer. Type Cult. Collec., 17th Edit., p. 21 (1987).

Hoelzle, I, et al., "of trehalose in Rhizobia Cultured Under 1% Oxygen", Appl. Environ. Micro., pp. 3213-3215, (1990).

Lame, L. et al. "Thermostable Amyulolytic Activity from Sulfolobus Solfataricus", BFE, vol. 8, No. 4, pp. 201-203, (1991).

Birch, G. "Trehaloses", Adv. in Carbo. Chem., Academic Press, pp. 202-225, (1963).

ART-UNIT: 161

PRIMARY-EXAMINER: Prats; Francisco

## ABSTRACT:

Disclosed are novel thermostable trehalose-releasing enzyme, and its preparations and uses. The enzyme is obtainable from the culture of microorganisms such as Sulfolobus acidocaldarius (ATCC 33909 and ATCC 49426) and Sulfolobus solfataricus (ATCC 35091 and ATCC 35092), and capable of hydrolyzing at a temperature of over 55.degree. C. the linkage between a trehalose moiety and the remaining glycosyl moiety in a non-reducing saccharide having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. Trehalose and compositions containing the same are extensively useful in food products, cosmetics and pharmaceuticals.

1 Claims, 5 Drawing figures





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L4: Entry 5 of 21

File: USPT

US-PAT-NO: 6150153

DOCUMENT-IDENTIFIER: US 6150153 A

TITLE: Thermostable trehalose-releasing enzyme

DATE-ISSUED: November 21, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ikegami; Shouji	Okayama			JP
Kubota; Michio	Okayama			JP
Sugimoto; Toshiyuki	Okayama			JP
Miyake; Toshio	Okayama			JP

US-CL-CURRENT: 435/252.1; 435/200, 435/201, 435/822

## CLAIMS:

We claim:

1. A biologically pure culture of a microorganism capable of producing a thermostable trehalose-releasing enzyme which specifically hydrolyzes the linkage between a trehalose moiety and the remaining glycosyl moiety in a non-reducing saccharide having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3, but which does not form trehalose from starch wherein said microorganism is selected from the group consisting of microorganisms of the genus Sulfolobus and mutants thereof, with the exclusion of Sulfolobus acidocaldarius strains having ATCC numbers 33909 and 49426, and Sulfolobus solfataricus strains having ATCC numbers 35091 and 35092, wherein said biologically pure culture is obtained by culturing said microorganism in a nutrient culture medium to produce said enzyme and removing the impurities from the culture to increase the purity of said enzyme, said biologically pure culture having at least 0.03 unit/ml of enzyme activity.

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L4: Entry 10 of 21

File: USPT

Feb 22, 2000

US-PAT-NO: 6027918

DOCUMENT-IDENTIFIER: US 6027918 A

TITLE: Recombinant thermostable enzyme which releases trehalose from non-reducing saccharide

DATE-ISSUED: February 22, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitsuzumi; Hitoshi	Okayama			JP
Kubota; Michio	Okayama			JP
Sugimoto; Toshiyuki	Okayama			JP

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Kabushiki Kaisha Hayashibara Seibutsu Kagaku Kenkyujo	Okayama			JP	03

APPL-NO: 08/ 798269 [PALM]

DATE FILED: February 11, 1997

## PARENT-CASE:

This is a division of co-pending parent application Ser. No. 08/505,377 filed Jul. 21, 1995.

## FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-190180	September 21, 1994
JP	7-109128	April 11, 1995
JP	7-189760	July 4, 1995

INT-CL: [07] C12 P 21/02, C12 N 9/24, C12 N 15/31, C12 N 15/52

US-CL-ISSUED: 435/69.2; 435/69.1, 435/71.1, 435/71.2, 435/183, 435/200, 435/252.33, 435/320.1, 536/23.2, 536/23.1, 536/23.7

US-CL-CURRENT: 435/69.2; 435/183, 435/200, 435/252.33, 435/320.1, 435/69.1, 435/71.1, 435/71.2, 536/23.1, 536/23.2, 536/23.7

FIELD-OF-SEARCH: 435/69.1, 435/69.2, 435/71.1, 435/71.2, 435/183, 435/252.33, 435/320.1, 435/200, 536/23.2, 536/23.1, 536/23.7

## PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

Search Selected

Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/>	<u>4521252</u>	June 1985	Miyake et al.	127/46.3
<input type="checkbox"/>	<u>5591612</u>	January 1997	Meruta et al.	435/100

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0 606 753 A2	December 1983	EP	
50-1544858	December 1975	JP	
58-23799	February 1983	JP	
58-72598	April 1983	JP	
58-216695	December 1983	JP	
2106912	August 1982	GB	

## OTHER PUBLICATIONS

Sambrook et al, Molecular Cloning: A Laboratory Manual; Cold Spring Harbour Laboratory Press; pp. v-xxxii; 1989.  
Laemmli, U.K., "Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4"; Nature; vol. 227; pp. 680-685; Aug. 15, 1970.  
The Amylase Research Society of Japan, editors; Handbook of Amylases and Related Enzymes: Their Sources, Isolation Methods, Properties and Applications; Pergamon Press; pp. xi-71; 1988.  
Licia et al., Thermostable Amylolytic Activity for Sulfolobus solfataricus; Biotech Forum Europe; vol. 8, No. 4, pp. 201-203; 1991.  
Reeck et al 1987 Cell 50 p 667, Aug 28, 1987.

ART-UNIT: 163

PRIMARY-EXAMINER: Knode; Marian C.

ASSISTANT-EXAMINER: Zeman; Mary K

## ABSTRACT:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 54,000-64,000 daltons and a pI of about 5.6-6.6, and releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3. The enzyme has a satisfactorily-high thermostability, i.e. it is not substantially inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of trehalose on an industrial scale and in a satisfactorily-high yield.

13 Claims, 6 Drawing figures

**WEST**

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L4: Entry 10 of 21

File: USPT

US-PAT-NO: 6027918

DOCUMENT-IDENTIFIER: US 6027918 A

TITLE: Recombinant thermostable enzyme which releases trehalose from non-reducing saccharide

DATE-ISSUED: February 22, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitsuzumi; Hitoshi	Okayama			JP
Kubota; Michio	Okayama			JP
Sugimoto; Toshiyuki	Okayama			JP

US-CL-CURRENT: 435/69.2; 435/183, 435/200, 435/252.33, 435/320.1, 435/69.1, 435/71.1, 435/71.2, 536/23.1, 536/23.2, 536/23.7

## CLAIMS:

We claim:

1. An isolated DNA molecule encoding a thermostable enzyme obtainable from a microorganism of the genus *Sulfolobus* having the following physico-chemical properties:

## (1) Action

Releasing trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3, but not substantially acting on maltooligosaccharides having a degree of glucose polymerization of at least 3;

## (2) Molecular weight

About 54,000-64,000 daltons on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDA-PAGE);

## (3) Isoelectric point (pI)

About 5.6-6.6 on isoelectrophoresis; and

## (4) Thermostability

Substantially not inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min.

2. The isolated DNA molecule according to claim 1, wherein a base sequence encoding said thermostable enzyme is selected from the group consisting of SEQ ID NO:2 and a sequence having sequence similarity to SEQ ID NO:2 where one or more bases in SEQ ID NO:2 are replaced with different bases on the basis of the degeneracy of the genetic code without altering the corresponding amino acid sequence of SEQ ID NO:1.

3. A replicable recombinant DNA molecule comprising a self-replicable vector and

an isolated DNA molecule according to claim 2.

4. A host cell transformed with a replicable recombinant DNA molecule according to claim 3.

5. The transformed host cell according to claim 4, which is *Escherichia coli*.

6. A process for preparing a recombinant thermostable enzyme, comprising the steps of:

culturing a host cell according to claim 4 to express and produce the recombinant thermostable enzyme; and

collecting the expressed and produced recombinant thermostable enzyme to prepare the recombinant thermostable enzyme.

7. The isolated DNA molecule according to claim 1, which is derivable from a microorganism of the genus *Sulfolobus*.

8. A replicable recombinant DNA molecule comprising a self-replicable vector and an isolated DNA molecule according to claim 1.

9. A host cell transformed with a replicable recombinant DNA molecule according to claim 8.

10. The transformed host cell according to claim 9, which is *Escherichia coli*.

11. A process for preparing a recombinant thermostable enzyme, comprising the steps of:

culturing a host cell according to claim 9 to express and produce the recombinant thermostable enzyme; and

collecting the expressed and produced recombinant thermostable enzyme to prepare the recombinant thermostable enzyme.

12. The replicable recombinant DNA as claimed in claim 8, wherein said self-replicable vector is plasmid vector Bluescript II SK(+).

13. The process as claimed in claim 11, wherein the produced recombinant thermostable enzyme is collected by one or more techniques selected from the group consisting of centrifugation, filtration, concentration, salting out, dialysis, separatory sedimentation, ion-exchange chromatography, gel filtration chromatography, hydrophobic chromatography, affinity chromatography, gel electrophoresis and isoelectrophoresis.

**WEST**

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L4: Entry 11 of 21

File: USPT

US-PAT-NO: 6017899

DOCUMENT-IDENTIFIER: US 6017899 A

TITLE: Non-reducing saccharide-forming enzyme, its preparation and uses

DATE-ISSUED: January 25, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maruta; Kazuhiko	Okayama			JP
Kubota; Michio	Osaka			JP
Sugimoto; Toshiyuki	Okayama			JP
Miyake; Toshio	Okayama			JP

US-CL-CURRENT: 514/53; 426/658, 435/100, 514/54, 514/61, 514/777, 514/778

## CLAIMS:

We claim:

1. A composition which consists essentially of

(i) a saccharide composition comprising

trehalose and non-reducing saccharides consisting of one or more glucose molecules bound to one trehalose molecule via the .alpha.-1,4 linkage or the .alpha.-1,6 linkage, where the total content of said non-reducing saccharides with two and three glucose molecules bound to the trehalose molecule is more than 43 w/w % but not more than 99 w/w %,

(ii) a member selected from the group consisting of foodstuffs, cosmetically acceptable materials, and pharmaceutically accepted materials,

whereby said saccharide composition is obtained by:

(a) contacting a solution containing reducing partial starch hydrolysates with an enzyme to form a non-reducing saccharide having a trehalose structure, said enzyme having the following physicochemical properties:

(1) Action

Converting at least one reducing partial starch hydrolysate having a degree of glucose polymerization of three or more to a non-reducing saccharide having a trehalose structure as an end unit;

(2) Molecular Weight

About 76,000-87,000 daltons on sodium dodecylsulfate-polyacrylamide gel electrophoresis;

(3) Isoelectric point

About 3.4-4.6 on isoelectrophoresis using ampholyte;

(4) Optimum Temperature

About 35-40.degree. C. when incubated at pH 7.0 for 60 minutes;

(5) Optimum pH

About 6.4-7.2 when incubated at 40.degree. C. for sixty minutes;

(6) Thermal Stability

Stable to a temperature of about 35-40.degree. C. when incubated at pH 7.0 for 60 minutes; and

(7) pH Stability

Stable at a pH of about 5.5-11.0 when incubated at 25.degree. C. for 16 hours;

(b) contacting the product from step (a) with glucoamylase or .alpha.-glucosidase to form trehalose; and

(c) collecting the resulting saccharide composition.

2. The composition of claim 1, wherein the step (b) further contains a step of crystallizing said trehalose.

3. The composition of claim 2, wherein said trehalose is hydrous- or anhydrous-crystalline trehalose.

4. The composition of claim 1, wherein the resultant mixture in the step (b) is further subjected to column chromatography using a strongly-acidic cation-exchange resin to increase the content of trehalose.

5. The composition of claim 1, wherein the trehalose structure in said non-reducing saccharide is located in its end unit.

6. The composition of claim 5, wherein said non-reducing saccharide having a trehalose structure is an .alpha.-glycosyl trehalose shown by the formula:

G.sub.n --T

wherein the symbol "G", "n", and "T" mean glucose, at least one integer and .alpha., .alpha.-trehalose residues, respectively.

7. The composition of claim 1, wherein said reducing partial starch hydrolysate is one or more reducing partial starch hydrolysates having a degree of glucose polymerization of 3 or higher.

8. The composition of claim 1, which is a food product.

9. The composition of claim 1, which is a cosmetic.

10. The composition of claim 1, which is a pharmaceutical.

11. A composition according to claim 1, wherein said enzyme is derived from a microorganism.

12. A composition according to claim 11, wherein said microorganism is a member selected from the group consisting of the genera Rhizobium, Arthrobacter, Brevibacterium, Flavobacterium, Micrococcus, Curtobacterium, Mycobacterium and Terrabacter, and mutants thereof.

13. A composition according to claim 1, wherein said enzyme has at least one partial amino acid sequence selected from the group consisting of:

(a) X.sub.1 -arginine-threonine-proline-X.sub.2  
-serine-threonine-tyrosine-arginine-leucine-(SEQ ID NO: 9), wherein the X.sub.1 is  
valine or methionine and X.sub.2 is alanine or valine;

(b) glycine-valine-glutamic acid-aspartic  
acid-threonine-alanine-phenylalanine-phenylalanine-arginine-tyrosine-(SEQ ID NO:  
6);

(c) leucine-valine-glutamine-leucine-threonine-methionine-proline-glycine-val  
ine-proline-(SEQ ID NO: 7); and

(4) glutamic acid-glycine-arginine-X.sub.3 -serine-X.sub.4  
-tyrosine-alanine-X.sub.5 -alanine-(SEQ ID NO: 10), wherein X.sub.3 is glycine or  
glutamine, X.sub.4 is proline or arginine, and X.sub.5 is valine or glutamic acid.

14. A composition according to claim 1, wherein said non-reducing saccharide is an  
.alpha.-glycosyl trehalose of the formula:

G.sub.n --T

wherein G, N, and T mean glucose residue, at least one integer, and  
.alpha., .alpha.-trehalose residue, respectively.



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TITLE: Recombinant thermostable enzyme which forms non-reducing saccharide from reducing amylaceous saccharide

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## PRIOR-ART-DISCLOSED:

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☐ Search Selected☐ Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/>	<u>4521252</u>	June 1985	Miyake et al.	127/46.3
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## ABSTRACT:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 69,000-79,000 daltons and a pI of about 5.4-6.4, and forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. The enzyme has satisfactorily high thermostability, i.e. it is substantially not inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of such non-reducing saccharides on an industrial scale and in a satisfactorily-high yield.

1 Claims, 6 Drawing figures